DOCKET NO.: PUAM-0259

PATENT

Application No.: Filed Herewith

Preliminary Amendment - First Action Not Yet Received

Amendments to the Specification:

1. On page 1, insert the following section prior to the "FIELD OF THE INVENTION" section:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of application serial no. 10/127,639, filed April 22, 2002, which is a divisional of application serial no. 09/241,862, filed February 2, 1999, which claims benefit under 35 U.S.C. §119(e) to provisional application serial no. 60/073,376, filed February 2, 1998. The disclosure of each of the foregoing patent applications is hereby incorporated by reference in its entirety.

2. Please amend the paragraph at page 8, spanning lines 7 - 10 as follows:

FIG. 2 (a). Double reciprocal plots of the initial rate data with [[UDP-GicNAc]] <u>UDP-GicNAc</u> as the varied substrate. Initial rates are measured at fixed acceptor 1b concentrations of 7 μ M (\diamond), 10 μ M (∇), 15 μ M (\blacksquare), 30 μ M (+), 100 μ M (\bullet). 0.08 μ M of purified MurG is used for each reaction.

3. Please amend the paragraph at page 8, spanning lines 15 - 23 as follows:

FIG. 3. [[IC50]] $\underline{IC_{50}}$ measurements for compound 12a and UDP. All the assays are performed under the same conditions with 18 μ M 1b and 34.3 μ M [[UDP-G1CNAc]] $\underline{UDP\text{-GlcNAc}}$. Each IC₅₀ value is determined by fitting five or six data points to equation:

$$v_{i} = \frac{1}{(1)}$$

$$v_{o} = 1 + IC_{50}$$

where v_i is the initial rate in the presence of inhibitor at concentration (1), and v_o is the initial rate without inhibitor.

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5. Please amend the paragraph at page 26, spanning lines 16 - 21 as follows:

After all of the amino acids are coupled, the pentapeptide is cleaved off of the resin by [[ishing]] washing with 1% TFA/CH₂Cl₂ (5 x 2 min, 15 mL each) with slight agitation. The cleavage solution is transferred via cannula into a vessel containing 2 mL of pyridine and 20 mL of methanol. The filtration is concentrated and purified three times by flash chromatography (5% MeOH/CHCl₃ with 1% AcOH) to give 300 mg (56%) of product. Rf 0.34 (10% MeOH/CHCl₃).

6. Please amend the paragraph spanning page 37, line 28 through page 38, line 7 as follows:

Compound 11a is made following the same scheme as 1a except that in step e, compound 6 is coupled to TEOC-NHCH₂CH₂NH₂ instead of to 7. The [[sily]] silyl protecting group is cleaved using TBAF, the same as in making 1a. R_f 0.20 (CHCl₃: MeOH: $H_2O = 3: 2: 0.5$); ¹H NMR (CD₃OD, 500 MHz) δ 5.58 (bs, 1 H), 5.11 (t, J = 7.0 Hz, 1 H), 4.30 (q, J = 6.7 Hz, 1 H), 4.21 (m, 1 H), 4.04 (m, 3 H), 3.72 (m, 1 H), 3.78 (m, 1 H), 3.73 (m, 1 H), 3.64 (m, 1 H), 3.50 (dd, J = 9.4, 9.4 Hz, 1 H), 3.40 (m, 1 H), 3.13 (m, 2 H), 2.03 (s, 3 H), 2.00 (m, 2 H), 1.73 (m, 1 H), 1.67 (s, 3 H), 1.63 (m, 1 H), 1.61 (s, 3 H), 1.46 (m, 1 H), 1.39 (m, 1 H), 1.38 (d, J = 6.7 Hz, 3 H), 1.18 (m, 1 H), 0.94 (d, J = 6.7 Hz, 3 H); ¹³C NMR (CD₃OD, 500 MHz) δ 176.2, 173.6, 131.2, 125.2, 95.6, 80.7, 78.1, 74.3, 70.3, 64.9, 62.0, 54.2, 39.7, 38.2, 37.8, 37.5, 29.8, 25.8, 25.2, 22.5, 19.1, 18.6, 17.0; HRMS(FAB) calcd for $C_{23}H_{43}N_3O_{13}P_2Na$ [M-2H⁺+Na⁺]: 654.2169, found 654.2199.

7. Please amend the paragraph at page 41, spanning lines 11 - 22 as follows:

To a microfuge tube containing 1 equivalent 1b (10 μ g) and 3 equivalents ¹⁴C-UDP-GlcNAc in 100 μ L HEPES reaction buffer (25 mM HEPES, pH 7.9, and 2.5 mM [[MgCl2]] MgCl₂) is added 1 μ g purified MurG. The reaction is terminated after 30 minutes by heating MurG to 65 °C for five minutes. The reaction is evaluated by transferring a 10 μ L aliquot to a tube containing a 3-fold molar excess of Tetralink Tetrameric Avidin Resin (based on the amount of 1b expected in one tenth of a volume of the reaction mixture), diluting with H₂O, transferring the suspension to a 96 well filter plate, and washing to remove

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unbound radioactivity as described in more detail under the experimental for the initial rate assays. The resin is then transferred to a scintillation vial containing Ecolite and counted. The conversion to disaccharide product 15 is estimated to be greater than 90% based on the counts incorporated into the resin. The mixture containing 15 is suitable for evaluating transglycosylase activity.